STUDIES ON CELL-DIFFERENTIATION: THE ROLE OF AUXIN IN ALGAE, WITH PARTICULAR REFERENCE TO RHIZOID-FORMATION IN BRYOPSIS [1]

WM. P. JACOBS

Biology Department, Princeton University, Princeton, New Jersey

Auxin, the plant growth-hormone, has been shown to occur in species of brown algae, red algae, green algae and diatoms (van der Weij, 1933; duBuy and Olson, 1937; van Overbeek, 1940a, 1940b; Thimann *et al.*, 1942). Judging by these reports auxin is ubiquitous in the algae, yet relatively few experiments in the literature deal with the possible role of auxin in these plants; the overwhelming majority of auxin-experiments have used seed-plant material. Most of the auxin-experiments on algae have been concerned with cell multiplication in unicellular Chlorophyceae. Leonian and Lilly (1937) found inhibition with 1 p.p.m. of indole-acetic acid (IAA) and toxic effects with 10 p.p.m. on the growth of five different species in medium containing sugar. Yin (1937) reported significant increases in average cell *diameter* of *Chlorella vulgaris* in the presence of 1 to 40 p.p.m. IAA and sugar, although he found "no conclusive differences (in) the total growth of the cultures as determined by . . . dry weight determinations." A "positive stimulating effect" of IAA and other growth-substances was reported in a brief note by Brannon (1937) for *C. vulgaris, C. pyrenoidosa* and an *Oocystis* sp. grown in sugar-free medium; he claimed to have "some evidence that growth substances accelerated the rate of cell reproduction and increased the size of cells." Pratt (1938), testing a wide range of IAA concentrations, found no effect on the average cell diameter of *C. vulgaris*, although the cell number per mm.^{3} was increased up to 32 times in sugar-free medium. Brannon's full report (Brannon and Bartsch, 1939) confirmed Pratt's finding of an increase in cell-number in *C. vulgaris* when IAA was added, but now reported that it was "impossible to secure reliable data respecting the effect of a growth substance upon the size of (*Chlorella*) cells." They pointed out that the inhibition from IAA reported by Leonian and Lilly (1937) was probably due to the use of dextrose in the culture medium, since Brannon and Bartsch found fewer cells per mm.^{3} with dextrose plus IAA than they did with dextrose alone. Lilly and Leonian (1941) complicated matters once more by reporting no increase in total dry weight of *Chlorella* cultures grown in the presence of 10 p.p.m. IAA but in the absence of dextrose. They state that there was a "fairly dependable parallelism" between dry weight and cell number. However, Brannon and Sell (1945) reported *increases* in dry weight of more than 4 times from the addition of the same concentration of IAA. Their culture of *Chlorella pyrenoidosa* was reportedly a duplicate of the culture called *C. vulgaris* by Lilly and Leonian (1941). Brannon and Sell obtained "pronounced and persistent variations" in preliminary experiments with IAA, variations which

[1] This work was carried out during the summer of 1950 while a Lalor Fellow at Woods Hole.

disappeared when the IAA was recrystallized to a constant melting point of 165° C.; they suggest lack of purification of IAA as a possible basis for the discrepant results of Lilly and Leonian. Inhibitory or toxic effects of IAA on cell number were reported by Manos (1945) for *C. vulgaris*. Unlike most of the other workers, Manos makes no mention of either adjusting the pH or of buffering.

An extensive paper by Algeus (1946) confirmed much of the earlier work and cleared up most of the discrepancies. The great importance of pH in IAA experiments was first pointed out: *Scenedesmus obliquus* and a strain of *C. vulgaris* showed an increase in cell number upon the addition of IAA only at acid pH, the effect of IAA increasing with decreasing pH. Furthermore, there were consistent differences in response to IAA not only between species but also between different strains of the same species. Only three of the ten species tested showed increases in cell number from any IAA concentration within the 0.1 to 100 p.p.m. range. For each of these three species the concentration giving maximum response was different. The observation of Brannon and Bartsch (1939) that IAA increased cell number in sugar-free medium but not in medium containing sugar was confirmed, as was Yin's (1937) report that IAA increased cell *size*. However, Algeus found cell size increased in sugar-free medium only at concentrations of IAA (100 p.p.m.) which were so high that the normal increase in cell number was inhibited. And only one of the two tested species showed even this increase.

In summary, a few species of unicellular green algae have been found to show an increase in cell number at IAA concentrations of the same order of magnitude as are effective physiologically in seed-plants. A larger number of unicellular green algae showed no increase in cell number even at acid pH, without added sugar, and with a wide range of applied IAA concentrations.

Auxin experiments with algae which are morphologically more complex have been even fewer. Olson and duBuy (1937) cited experiments to show that the polarity of the germinating zygote of *Fucus* could be determined by a high local concentration of IAA. They found no increase in the number of rhizoids formed when IAA was added. Young *Bryopsis* plants were treated with IAA in sea water at various molar concentrations by Darsie (1939), but the only effect observed was inhibition of growth at the 10^{-3} *M* and 10^{-4}*M* concentrations. It should be mentioned, however, that throughout his work Darsie deliberately restricted his attention to growth and tropisms of the shoot only. Kylin (1942) found that IAA increased the number of cells formed by germinating zygotes of *Ulva* and zoospores of *Enteromorpha* at pH 8.2, a result that has been confirmed with respect to *Ulva* by Levring (1945). Davidson (1950) applied a range of concentrations of various growth substances to fronds of *Fucus* and *Ascophyllum* and to *Fucus* sporelings. He reported stimulation of terminal frond growth, except with indole-butyric acid on *Ascophyllum* fronds, and stimulation of general growth of *Fucus* sporelings with IAA, indolebutyric and naphthalene-acetic acids. However, auxins in lanolin usually inhibited frond growth and never stimulated it. Auxins had no stimulatory effect on intercalary growth of fronds of either genus, no matter what the concentration or method of application. The fact that the "inhibitor" iodoacetic acid stimulated *Fucus* sporelings in essentially the same pattern relative to concentration as did IAA suggests the advisability of controlling pH effects. This was not done.

Williams (1949) reported that IAA produced gains in wet weight of small discs cut from some portions of the blade in *Laminaria*. Here, too, the published

results are not unequivocal. There were no replications, no measures of variability; almost half of the IAA-treated samples disintegrated during the 16-day experimental period, and the largest wet-weight increase reported was only 18 per cent over the controls.

It appears, then, that while auxin is widely present in the algae, there is little evidence as to its role—if any—in algal morphogenesis.

It is reasonable to expect auxin to have somewhat similar roles in the various plant groups in which it occurs. Now, one of the most striking effects of auxin in flowering plants is the stimulation of root formation by high concentrations of auxin. The structure in algae which most closely corresponds to the root of a flowering plant is the rhizoid. Although the rhizoid is a mere protuberance from a single cell (instead of a multicellular organ with a very high degree of cellular differentiation and diversity like the root) yet the rhizoid is usually negatively phototropic, forms a close anchoring connection with the substratum, and is formed typically at the basal end of stem-like structures. These are characteristics which rhizoids share with roots. Hence, the following experiments were designed to determine if rhizoid-formation in algae is stimulated by auxin.

Materials and Methods

Bryopsis plumosa (Hudson) C. Agardh was used as experimental material. *Bryopsis* was chosen because its normal life cycle is well known, some aspects of its developmental physiology and morphology have been studied (Noll, 1888; Winkler, 1900; Steinecke, 1925; Darsie, 1939), it has already been shown to contain relatively large amounts of ether-extractable auxin (Darsie, 1939; van Overbeek,

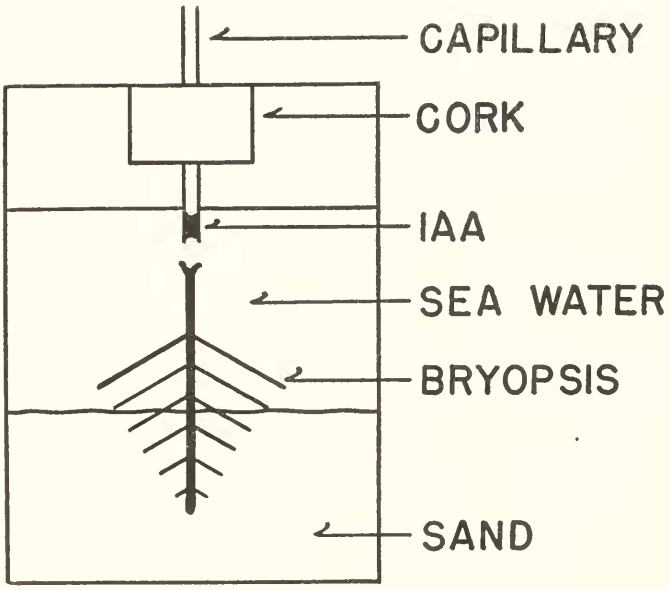

Figure 1. Diagram to show method of establishing a gradient of IAA along the long axis of *Bryopsis* plants.

1940a) and in its unicellular, multinucleate structure it seemed satisfactorily far removed from the organization of a flowering plant (Fritsch, 1935).

The general morphology of a young plant is shown diagrammatically in Figure 1. The vertically upright stem is positively phototropic, forms branches at the apex and negatively phototropic rhizoids at its base (Winkler, 1900; Steinecke, 1925). The chloroplasts are typically concentrated in the illuminated portions of the plant. The lumen of a young branch is in continuity with that of the main axis but by the time a branch is the 18th to 20th branch from the apex a cross-wall is beginning to form at its base. Thus, the branches furthest from the stem apex are morphologically separate cells.

Freshly collected, vigorous plants of *B. plumosa* were examined under the dissecting microscope and paired on the basis of close similarity in size, number of branches and rhizoids. One plant of each pair was placed in an inverted position in a shell-vial containing 10 ml. of sea water, as shown in Figure 1. The distal half of the branch-bearing portion was embedded in sand. A glass capillary was held so that the lower end was a few mm. above the base of the plant. The lower end of the capillary was filled with 0.7 to 1.0 mg. of IAA crystals. In replications of the experiment which used longer-stemmed *Bryopsis* plants, the lower opening of the capillary was held about 1 cm. above the oldest branches (*i.e.*, half-way down the unbranching portion of the main stem). The shell-vials shown in Figure 1 were placed in a water bath (at 21.5 ± 1° C.) to cut down convection currents which would disturb the diffusion gradient of IAA from the capillary tube. The relatively dim light from the windows was supplemented during the day with a 60 watt incandescent lamp held 18–24 inches above the shell-vials. The experiments lasted 12–15 days. Progressive dissolving of the IAA crystals occurred during the experimental period. There were usually some crystals left in the capillary even after 12 days.

To determine the extent to which the acid IAA changed the pH of the sea water, acetic acid was added to sea water to give the same molar concentration as was obtained with the highest concentration of IAA used (1.0 mg./10 ml. of sea water). (Acetic acid and IAA have the same pK.) The pH determinations were made with a Cambridge pH meter using a glass electrode. The untreated sea water had a pH of 8.01; addition of the acetic acid gave a pH of 7.25. Since all the IAA in the capillary was rarely dissolved by the end of each experiment and since the total possible drop in pH was so relatively small, pH effects were hereafter neglected.

At the end of the experiment the plants were first examined *in situ* with a horizontal microscope, then removed and compared under higher powers of the dissecting microscope.

Results and Discussion

When small, independently growing plants were prepared as described above, the results of all six replications showed that the superposition of an auxin gradient induces rhizoid-formation at the base of 77–95 per cent of the exposed (*i.e.*, above-ground) branches, while no rhizoids are formed at the base of branches in the control plants (Table I). Those branches which were below the level of the sand showed no basal rhizoids in either group of plants.

As *Bryopsis* plants continue their growth, secondary branches grow out on the primary branches and rhizoids usually develop at the base of the primary branches

near their region of connection with the main stem. Eighteen of these primary branches with their array of secondary branches were dissected off the original main stem, paired and then treated as were the individual plants above. Results are shown in Table II. The data indicate that the auxin-gradient still has a stimulatory effect on rhizoid-formation although the secondary branches are not nearly as reactive as are the young primary branches.

These experiments verify the hypothesis that the external application of auxin acts in *Bryopsis* to induce rhizoids at the region of relatively high local concen-

TABLE I

Effect of an external auxin-gradient on rhizoid formation in young Bryopsis plants. (The results of two of the six replications are shown.)

IAA-treated		Controls	
No. of exposed branches	No. of branches forming rhizoids	No. of exposed branches	No. of branches forming rhizoids
13	10	24	0
19	18	20	0

tration. The more extended hypothesis that auxin is the agent, naturally occurring in *Bryopsis*, which controls rhizoid-formation in the normal untreated plant is also supported by our experiments, particularly when they are considered in the light of reports of ether-extractable auxin in *Bryopsis* (Darsie, 1939; van Overbeek, 1940a) and of Darsie's finding that 73 per cent of the total plant auxin is in the basal (*i.e.*, rhizoid-forming) half of single-celled *Bryopsis* plants.

A possible obscuring factor was that despite the surface cleaning done under the dissecting microscope, the *Bryopsis* plants were, of course, not sterile; hence, the possibility must be recognized that the treatment with auxin may have acted indirectly on *Bryopsis* through the other organisms present. In my opinion this is unlikely.

The following interpretation of the development of *Bryopsis* can be advanced, based on these studies and those of previous researchers. *Bryopsis* is an organism

TABLE II

Effect of an external auxin-gradient on rhizoid formation by secondary branches of isolated primary branches

No. of pairs with more rhizoids on auxin-treated branches	No. of pairs with the same no. of rhizoids on each	No. of pairs with more rhizoids on the control branches
6	3	0

in which cytoplasm and chloroplasts can easily migrate from one portion of the coenocyte to another (Winkler, 1900; Steinecke, 1925). In the normal young plant auxin collects at the basal end of the coenocyte (Darsie, 1939) and there induces the formation of rhizoids. Once the older primary branches become morphologically separated from the main stem by the formation of thick cross-walls at their proximal end, the auxin produced in these branches can much less readily, if at all, move into the main stem. When the concentration of auxin becomes high enough at the proximal end of these separate branches rhizoids are formed there. Proximal rhizoids were never found in freshly collected material except when the "isolating" cross-wall was already present.

When *Bryopsis* is shaded (Winkler, 1900) the branch-tips continue their elongation as rhizoids instead of as "shoot" structures. Winkler (1900) and Steinecke (1925) have reported experiments which they believe show that this transformation is preceded by a migration of chloroplasts and cytoplasm out of the branch-tip and their replacement by cytoplasm which was in the rhizoidal end of the coenocyte. The experiments with auxin suggests that hormonal redistribution accompanies or is a consequence of the redistribution of cytoplasm.

Hence, it is suggested that one of the key differences between *Bryopsis* and seed-plants, the difference which is reflected in the strict developmental polarity of seed-plants and the labile polarity of *Bryopsis*, is that seed-plants have a strongly polar system for auxin-transport (Went and Thimann, 1937; Jacobs, 1950) while *Bryopsis* does not.

Summary

1. Application of indole-acetic acid, a naturally occurring auxin, to young *Bryopsis* plants results in the differentiation of rhizoids at the proximal end of most of the exposed "branches." Control plants formed no basal rhizoids.

2. Similar results were obtained with respect to rhizoid formation on secondary "branches" when the primary branches were excised from the original main stem and exposed to auxin. The response was not as strong under these circumstances.

3. Considered with the fact that auxin is present in large amounts in *Bryopsis*, with most of the auxin being in the lower (*i.e.*, rhizoid-forming) half of the plant, these experiments verify the hypothesis that the formation and distribution of auxin control rhizoid-formation in *Bryopsis* in a closely analogous way to auxin's effect on root-initiation in seed-plants.

LITERATURE CITED

Algeus, S., 1946. Untersuchungen über die Ernährungsphysiologie der Chlorophyceen. Mit bensonderer Berücksichtigung von Indolylessigsäure, Ascorbinsäure und Aneurin. *Bot. Notiser*, 129–280.

Brannon, M. A., 1937. Algae and growth-substances. *Science*, **86**: 353–354.

Brannon, M. A., and A. F. Bartsch, 1939. Influence of growth substances on growth and cell division in green algae. *Amer. J. Bot.*, **26**: 271–279.

Brannon, M. A., and H. M. Sell, 1945. The effect of indole-3-acetic acid on the dry weight of *Chlorella pyrenoidosa*. *Amer. J. Bot.*, **32**: 257–258.

Darsie, M. L., 1939. Certain aspects of phototropism, growth and polarity in the single-celled marine alga, Bryopsis. Ph.D. Dissertation, Stanford University.

Davidson, F. F., 1950. The effects of auxins on the growth of marine algae. *Amer. J. Bot.*, **37**: 502–510.

DuBuy, H. G., and R. A. Olson, 1937. The presence of growth regulators during the early development of Fucus. *Amer. J. Bot.*, **24**: 609–611.

Fritsch, F. E., 1935. The structure and reproduction of the algae. Vol. I. Macmillan Co., New York.

Jacobs, W. P., 1950. Auxin-transport in the hypocotyl of *Phaseolus vulgaris* L. *Amer. J. Bot.*, **37**: 248–254.

Kylin, H., 1942. Über den Einfluss von Glucose, Ascorbinsäure und Heteroauxin auf die Keimlinge von *Ulva* und *Enteromorpha*. *Fysiografiska Sällsk. i Lund, Förh. Kgl.*, **12**: 135–148.

Leonian, L. H., and V. G. Lilly, 1937. Is heteroauxin a growth-promoting substance? *Amer. J. Bot.*, **24**: 135–139.

Levring, T., 1945. Some culture experiments with *Ulva* and artificial sea water. *Fysiografiska Sällsk. i Lund, Förh. Kgl.*, **16**: 45–56.

Lilly, V. G., and L. H. Leonian, 1941. Some factors affecting the dry weight of *Chlorella vulgaris*. *Amer. J. Bot.*, **28**: 569–572.

Manos, E., 1945. The effect of heteroauxin on the growth of Chlorella. *Biol. Rev. Coll. City of New York*, **7**: 11–15.

Noll, F., 1888. Über den Einfluss der Lage auf die morphologische Ausbildung einiger Siphoneen. *Arb. Bot. Inst. Würzburg*, **3**: 466–476.

Olsen, R. A., and H. G. DuBuy, 1937 The role of growth substance in the polarity and morphogenesis of Fucus. *Amer. J. Bot.*, **24**: 611–615.

Pratt, R., 1938. Influence of auxins on the growth of *Chlorella vulgaris*. *Amer. J. Bot.*, **25**: 498–501.

Steinecke, F., 1925. Zur Polarität von Bryopsis. *Bot. Archiv*, **12**: 97–118.

Thimann, K. V., F. Skoog and A. C. Byer, 1942. The extraction of auxin from plant tissues. II. *Amer. J. Bot.*, **29**: 598–606.

Van der Weij, H. G., 1933. On the occurrence of growth substance in marine algae. *Proc. Kon. Akad. Wetensch. Amsterdam*, **36**: 759–760.

Van Overbeek, J., 1940a. Auxin in marine algae. I. *Plant Physiol.*, **15**: 291–299.

Van Overbeek, J., 1940b. Auxin in marine algae. II. *Bot. Gaz.*, **101**: 940–947.

Went, F. W., and K. V. Thimann, 1937. Phytohormones. Macmillan Co., New York.

Williams, L. G., 1949. Growth regulating substances in *Laminaria agardhii*. *Science*, **110**: 169.

Winkler, H., 1900. Ueber Polarität, Regeneration und Heteromorphose bei Bryopsis. *Jahrb. wiss. Bot.*, **35**: 449–469.

Yin, H. C., 1937. Effect of auxin on *Chlorella vulgaris*. *Proc. Nat. Acad. Sci.*, **23**: 174–176.